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Processing and characterization of durum wheat bread enriched with antioxidant from yellow pepper flour

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ABSTRACT

The effect of the addition of yellow pepper flour on bread physico-chemical and sensorial properties was addressed in this study. In particular, vegetable flour concentration was set at 25%; in order to optimize the bread sensorial properties, yellow pepper flour was separately hydrated at three different water content levels. Texture analysis were carried out on both dough and bread samples to evaluate their firmness. Furthermore, tomographic analysis was performed on the same samples in order to provide a more detailed view of their texture. Estimation of the glycemic response, determination of the carotenoids content and sensory analysis of the fortified bread were also determined. Results highlighted that the highest glycemic index was achieved in bread sample having the highest water content and that showed the worst results in terms of texture. Among the studied samples, bread with medium hydration level showed good structural characteristic, double anti-oxidant content compared to the control bread (CTRL S) and the highest sensorial quality.

1. Introduction

Bread is a food produced using simple ingredients such as wheat flour, salt, yeast and water, but despite this, is one of the most consumed cereal products in many countries and a food at the basis of the diet of many people around the world. Precisely because of its simplicity and its wide consumption, the bread is suitable to be enriched and fortified with ingredients that can bring benefits to the consumer in terms of health. In fact, nowadays consumers prefer to eat healthier foods in order to prevent non-communicable diseases (Hathorn, Biswas, Gichuhi, & Bovell-Benjamin, 2008).

Among the ingredients that could be included in bread formulation there are vegetables, which are important part of the human diet. Pepper (*Capsicum annum*) is a vegetable of the Solanaceae family, native of South America, whose cultivation has spread around the world. Its fruits are rich in vitamins, mineral salts, and carotenoids (a class of antioxidants primarily found in yellow or red vegetables able to neutralize free radicals in cell membranes) such as beta-carotene, lutein and capsantin (Holmes & Kemble, 2009; Mateos et al., 2003) and other substances beneficial to health. So the incorporation of yellow pepper flour would improve the nutritional value of bread. Current researches have confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases, cancers and neurodegenerative diseases, as well as inflammation and problems caused by cell and cutaneous aging (Fan, Zhang, Yu, & Ma, 2006). Studies have been carried out to find potential sources of natural carotenoids in food, in particular, as pointed out by Hidalgo, Brandolini, and Pompei (2010), durum wheat flour used for the production of bread, baked products and pasta, provides a significant carotenoid contribution to the human diet. Because of the high levels of carbohydrates in bread, the determination of glycemic index (GI) of yellow pepper flour enriched bread seemed to be an important criterion to take into account when evaluating the so-called nutritional and physiological advantages of this product. According to the definition given by the Food and Agriculture Organization/World Health Organization (FAO/WHO, 1998), this index corresponds to the incremental area under the blood glucose response curve of a 50 g carbohydrate portion of a test food expressed as a percentage of the response to the same amount of carbohydrate from a standard food taken by the same subject (FAO/WHO, 1998).

The knowledge information regarding the effects of vegetable flours on dough and bread physico-chemical properties are few. Pumpkin seed products were incorporated into wheat flour to

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manufacture fortified bread (El-Soukkary, 2001; Giami, Mepba, Kiin-Kabari, & Achinewhu, 2003). The effect of the vegetable ingredients on the dough and bread quality as well as on the nutritional composition of the obtained bread was evaluated. Also Ptitchkina, Novokreschonovaa, Piskunova, and Morris (1998) explored the effect of addition of pumpkin powder to a standard wheat bread formulation. The most recent study is that of Mastromatteo, Danza, Guida, and Del Nobile (2012b), where the manufacturing of vegetable flour loaded bread was optimized acting on the flour hydration process. However, due to the high temperature of the drying process, the vegetable flour used had a low carotenoid content, as also highlighted by Padalino, Mastromatteo, Lecce, Cozzolino, and Del Nobile (2013). This may represent a serious limitation to the wide use of vegetable flour fortified bread.

In this direction, the aim of the work was the manufacturing of durum wheat bread added with yellow pepper flour obtained by means of a mild drying process. In particular, the production methodology proposed by Mastromatteo et al. (2012b), was used in this study to obtain a carotenoids-enriched durum wheat bread with good physico-chemical and sensorial properties. To this aim the following analysis were run: textural analysis of the dough samples and the manufactured bread, sensorial analysis, estimation of the glycemic response and determination of the carotenoids content.

2. Materials and methods

2.1. Raw materials

Durum wheat flour was bought from Tandoi mill (Molini Tandoi S.p.A., Corato, Bari, Italy), yellow pepper flour was purchased from Farris farm (Troia, Foggia, Italy), whereas guar seed flour was supplied from Farmalabor s.r.l (Canosa di Puglia, Bari, Italy). Fresh compressed yeast, salt and extra virgin olive oil were bought from a local market, dried sourdough was supplied from Bongiovanni mill (Molini Bongiovanni S.p.A., Villanova Mondovi', Cuneo, Italy). The fresh vegetable, the yellow pepper, was subjected at a mild drying process, in particular it was dried at constant temperature of 65 °C for 460 min. The moisture content of the fresh vegetable was about 90 g moisture/g dry matter, whereas that of the yellow pepper flour after the mild drying process was about 13 g moisture/g dry matter. Moreover, the vegetable flour presented for the centesimal composition, g/100 g dry matter, respectively: protein 3.1; soluble dietary fiber 8.7; insoluble dietary fiber 13.3; total dietary fiber 22.1. The water absorption capacity of the yellow pepper flour is about 35%.

2.2. Breadmaking process

Dough mixing, processing and baking were performed on laboratory-scale equipment. Durum wheat bread formulated without yellow pepper flour was used as first reference sample (CTRL S); while a second reference sample consisted of durum wheat bread with the addition of no-hydrated yellow pepper flour (CTRL P). The breadmaking process parameters and the ingredients amount were chosen according to a well-defined recipe followed by the same authors in a previous study (Mastromatteo, Danza, Guida, & Del Nobile, 2012a, 2012b). Regarding the other investigated bread samples, yellow pepper flour previously hydrated by using different amounts of hot water (0.4, 0.7, 1.0 L) was added to the dough formulation in order to obtain another three bread samples named as P-0.4, P-0.7, P-1.0. Also in this case, the recipe and the breadmaking process were the same used in a previous work (Mastromatteo et al., 2012a, 2012b).

After baking, bread samples were cooled down for about 2 h at room temperature and were submitted to instrumental, chemical and sensory analyses. Baking process was performed in triplicate. All the dough samples investigated are listed in Table 1.

2.3. Textural properties

2.3.1. Dough texture analysis

The tensile properties of the investigated doughs were measured by using a Texture Analyzer Zwick/Roell model Z010 (Zwick Roell Italia S.r.l., Genova, Italia) equipped with a dough tensile testing device. Dough samples for the texture analysis were prepared as those used in breadmaking process without adding any yeast to the formulation to avoid bubble interference. Before beginning the analysis, the material to be tested was placed between the molding and compression plates, so that inside the press samples with suitable size analysis were formed. After this stage, each sample was individually resting on a support table, which was inside the materials testing machine. The material testing machine starts in the tensile direction and the tensile hook recorded the test load. Pre-load of 0.01 N, load cell of 50 N and crosshead speed constant of 50 mm/min were the trial specifications.

2.3.2. Crumb texture analysis

All bread loaves were uniformly sliced to a thickness of 15 mm and the loaf crust was cut off allowing only crumb texture measurements. Cylindrical crumb samples (280 mm diameter) were cut from the center of each bread loaf using a circular cutter. Compression tests were carried out by using a Texture Analyzer Zwick/Roell model Z010 (Zwick Roell Italia S.r.l., Genova, Italia). An insert plate fixed in the universal work platform (100 × 90 × 9 mm) and compression die (75 mm diameter) were the parallel plates inside which the cylindrical breadcrumb samples were placed. The force required to compress slices of bread to a predetermined level of penetration against a rigid back plate using a cylindrical plunger was recorded for each sample tested. Pre-load of 0.3 N, load cell of 1 kN, maximum percentage deformation of 50% and a constant crosshead speed of 100 mm/min were the experimental conditions.

2.4. Tomographic analysis

For X-ray microtomographical analysis (μCT) the dough and bread samples were imaged under the same conditions, using the Skyscan 1172 high-resolution desktop X-ray microtomography system (Skyscan, Belgium). The dough samples were analyzed after 105 min of leavening but, in order to inactivate the yeast and therefore avoid the continuous rising of the dough during scanning, the samples were placed in cold storage (4 °C) for 20 min. In both cases, dough and bread samples were prepared as those used in

Table 1
Formulations of the investigated bread samples.

Sample	[a]Semolina flour (g/100 g)	[a]Yellow pepper flour (g/100 g)	[a]Guar Gum (g/100 g)	[b]Water content (L)	Total water content (L)
CTRL S	100	–	–	–	2.9
CTRL P	75	25	2	–	2.9
P-0.4	75	25	2	0.4	3.3
P-0.7	75	25	2	0.7	3.6
P-1.0	75	25	2	1.0	3.9

[a] g/100 g flour basis.
[b] Water content used to hydrate the vegetable flour before the breadmaking process.

breadmaking (they contained the amount of water specified in Table 1) and were placed on a round plate; the source and the detector were fixed, while the sample was rotated during measurement. Power settings of 100 kVp and 100 μA were used. A CCD camera with 2000 × 1048 pixels was used to record the transmission of the conical X-ray beam through all samples. The distance source-object-camera was adjusted to produce images with a pixel size of 2 μm. Four-frame averaging, a rotation step of 0.40° and an exposure time of 1767 ms were chosen to minimize the noise, covering a view of 180°. Scan time, on average, required 30 min. A set of flat cross section images was obtained for each sample after tomographical reconstruction by the reconstruction software NRecon (Skyscan). For image processing and analysis the skyscan software, CT-Analyzer (CTAn) was used. For data analysis, prior to 3D reconstruction, a component-labeling algorithm, available within CTAn, was used to isolate the largest 3D connected structures. The following four geometric parameters were measured using the CTAn software (Skyscan): Percent object volume (POV), Object surface/volume ratio (OSVR), Fragmentation index (FI), Structure Thickness (St.Th) and Structure Seperation (St.Sp) (Laverse, Mastromatteo, Frisullo, & Del Nobile, 2011).

2.5. Sensory analysis

Bread samples were submitted to a panel of 10 trained tasters (four men and six women, aged between 28 and 45) in order to evaluate the sensorial attributes. The panelists were selected on the basis of their sensory skills (ability to accurately determine and communicate the sensory attributes as appearance, odor, flavor and texture of a product). The panelists were also trained in sensory vocabulary and identification of particular attributes by evaluating durum wheat commercial bread. Loaf samples were sliced with an electric slicing knife (Atlantic S.p.A., Calenzano, Firenze, Italy) without removing the crust. Each sample was placed on white plates and identified with random three-digit numbers. The bread samples were evaluated for attributes such as color, appearance, crust and crumb firmness, large bubbles and overall quality by using a 9-point scale (Mastromatteo et al., 2012a, 2012b; Petitot, Boyerb, Minierb, & Valérie Micard, 2009).

2.6. Carotenoid determination

2.6.1. Chemicals

All chromatographic solvents were high-performance chromatography (HPLC) ultra-gradient grade and were purchased from Carlo Erba Reagents (Milan, Italy). Ammonium acetate was purchased from Sigma Aldrich (Milan, Italy). β-carotene, lutein and zeaxanthin purchased from Extrasynthese (Genay Cedex, France) assay ≥95%.

2.6.2. Extraction method

The carotenoids were extracted as described by Sun et al. (2007) with slight modifications. 10 g of yellow pepper flour were mixed with 50 mL of dichloromethane and the mixture was gently stirred at 35 °C for 20 min. The extracts were centrifuged at 5000 × *g* for 10 min at 4 °C (Eppendorf 5804 R, Milan, Italy) and the supernatant was transferred to a clean flask. The residue was mixed with another 50 ml of dicloromethane to repeat the extraction. The resulting supernatant was combined with the previous one. This operation was repeated 5 times. The combined supernatants were evaporated to dryness under vacuum at 35 °C and the residue was dissolved in 3 ml of dicloromethane, filtered through a 0.45 μm syringe filter (Teknokroma PTFE 0.45-μm) and then used for HPLC analyses.

2.6.3. High-performance liquid chromatography

The carotenoids were separated and quantified by HPLC as described by Sun et al. (2007). The HPLC used was an Agilent 1200 apparatus (Agilent Technologies Inc, Santa Clara, USA) consisting of an LC ChemStation 3D system controller, degasser, binary pump solvent delivery, auto sampler, column oven and DAD detector system was used. The column used for this separation was C_{18} Aqua 5 μ 200 A (150 × 2.00 mm) and 5 μm particles diameter (Phenomenex, Milan, Italy). Ten μL samples of extract or calibration standards were injected directly into the column. The mobile phase consisted of 30% ammonium acetate 1 mol/L in methanol (eluant A) and methanol (eluant B). The elution program was as following: at 0 min 5% B, 25 min 95% B, 40 min 95% B at the flow rate of 0.5 ml/min. Detection was performed by monitoring the absorbance signals at 450 nm. The retention times of carotenoids were identified using the UV–visible spectra of pure reference standards. The extractions were carried out in duplicate and analyses were carried out in triplicate. The calibration curves obtained by injecting standard solutions containing β-carotene, lutein and zeaxanthin were characterized by a correlation (r^2) > 0.988. The same procedure reported above for carotenoide evaluation in the flour was also applied for the extraction and the separation of carotenoids in the bread before and after cooking.

2.7. Glycemic response of breads

2.7.1. In vitro digestion

The digestion was carried out as described by Chillo, Ranawana, and Henry (2011) with slight modifications. Briefly bread (5 g) were tipped into a digestion vessel with 50 ml of distilled water and 5 ml maleate buffer (0.2 mol/L pH 6.0, containing 0.15 g $CaCl_2$ and 0.1 g sodium azide per liter) in a block at 37 °C (GFL 1092, Germany) and allowed to equilibrate for 15 min. Digestion was started by adding 0.1 ml amyloglucosidase (A 7095 Sigma Aldrich, Milan, Italy) and 1 ml of 2 g/100 g pancreatin (P7545 Sigma Aldrich, Milan, Italy) in quick succession and the vessel were stirred at 130 rpm. At 0, 20, 60, and 120 min 0.5 ml of digested samples was removed for analysis of released glucose. After the 120 min sampling the digests were homogenized using an Ultraturrax (Ika, Staufen, Germany) to convert them into slurries. The incubation continued for 1 h and 0.5 ml of digested samples was removed for analysis of released glucose.

2.7.2. Analysis of starch digest

The samples removed during digestion were added to 2.0 ml of ethanol and mixed. After 1 h, the ethanolic subsamples were centrifuged (2000 × *g*, 2 min) (Biofuge fresco HERAEUS, Germany) and an aliquot (0.05 ml) of the supernatant was removed. This aliquot was added to 0.25 ml amyloglucosidae (E-AMGDF, Megazyme International Ireland Ltd, 1 ml/100 ml in sodium acetate buffer 0.1 mol/L, pH 5.2) for 10 min at 20 °C. 0.75 ml DNS solution (10 g 3,5-dinitrosalcylic acid, 16 g NaOH and 300 g Na–K tartarate (Sigma Aldrich, Milan, Italy) made to final volume 1 L) was then added to the tubes. The tubes were heated for 15 min in boiling water, then cooled in cold water for 1 h, after which 4 ml of water (15 °C) was added. After mixing, the reducing sugar concentration was measured colorimetrically (530 nm) using a Shimadzu UV–vis spectrophotometer (model 1700, Shimadzu corporation, Kyoto, Japan). Glucose standards of 10.0 mg/ml were used. The results were then plotted as glucose release (mg) per g of sample vs time.

2.8. Statistical analysis

The experimental data were subjected to statistical evaluation using a one-way variance analysis (ANOVA). Duncan's multiple range tests were used to determine the difference among means and the significance was defined at $p < 0.05$. To this aim an STATISTICA 7.1 for Windows (StatSoft, Inc, Tulsa, OK, USA) was used.

3. Results and discussion

3.1. Texture analysis

3.1.1. Dough texture analysis

Texture analysis results performed on the dough samples are presented in Table 2. The dough properties were evaluated by means of two parameters, the maximum strain ($Strain_{max}$) and the break strain ($Strain_{break}$). The first expresses the deformation at the maximum force while the second allows to evaluate the deformation corresponding with the sample rupture. Regarding the reference samples, CTRL P and CTRL S, significantly different values of the investigated parameters were observed. In fact, they recorded respectively the lowest and the highest values of both $Strain_{max}$ and $Strain_{break}$. In particular, there was a loss of elasticity of the dough attributable to the addition of no-hydrated yellow pepper flour (CTRL P) compared with the sample produced using only durum wheat flour (CTRL S).

The dough weakening could be due to: (a) the decrease in wheat gluten because of the dilution effect and (b) the competition between proteins of the vegetable flour and wheat flour for water (Deshpande, Rangnekor, Sathe, & Salunkhe, 1983). These results also agree well with those of Ranga Rao, Haridas, Kumar, and Shurpalekar (1980), who reported that supplementation of wheat flour with 5–20% wheat germ, on weight basis, decreased water absorption, stability and softening of bread dough. They also agree with those of Mansour, Dworschak, Pollhamer, Gergely, and Hovari (1999).

These results are also confirmed by the higher values of maximum and break force (F_{max} and F_{break}) (Table 2) of the CTRL P sample compared to the CTRL S sample, which prove the higher level of hardness of the sample enriched with no-hydrated yellow pepper flour. Results are in agreement with what it was expected. In fact, the behavior of the reference samples is similar to that observed in a previous work (Mastromatteo et al., 2012b). As found by Mastromatteo et al. (2012b), the elasticity of the dough decreased with the addition of no-hydrated vegetable flour to bread formulation. Data listed in Table 2 also highlight that there were significant differences between the samples in which the yellow pepper flour and durum wheat semolina were separately hydrated. In particular, the P-0.4 and P-1.0 samples, having respectively the lowest and the highest level of hydration, were both different from the sample hydrated with intermediate water content (P-0.7). In addition, they shown (P-0.4 and P-1.0 samples) no statistically significant differences with the reference sample CTRL P; unlike the P-0.7 sample, which had a behavior closer to that of the reference sample CTRL S. Regarding the F_{max} and F_{break} (Table 2), data show that their values decreased with the increase of the water content, in particular the P-0.7 and P-1.0 samples recorded statistically similar values, which were found to be lower than that of the other investigated samples. What reported beforehand is in agreement with what found in a previous work (Mastromatteo et al., 2012b), the method used to hydrate durum semolina and yellow pepper flour strongly affects the dough tensile properties. In addition, this study corroborates the fact that hydrated vegetable flour avoid the competition for water between durum wheat and yellow pepper flours, promoting the formation of a better dough structure. The water absorption capacity by the dough was increased significantly due to the addition of hydrated yellow pepper flour to the formulation; this was in agreement with those reported by El-Soukkary (2001), who studied the evaluation of pumpkin seed products for bread fortification.

Bread dough has properties that differ from each other according to different water content. If the water is insufficient for the hydration of all dough ingredients, the gluten does not become fully hydrated and the elastic nature of the dough does not become fully developed. Conversely, an excessive level of free water in the dough results in the domination of the viscous component of dough, with a decreased resistance to extension, increased extensibility and the development of sticky dough (Spies, 1997, pp. 343–361). Specifically, the water added to the flour fulfills four functions; it dissolves soluble molecules, activates enzymes, brings about the formation of new bonds between the macromolecules in the flour, and alters the rheological properties of the dough. The physical state of water in food systems is believed to play an important role in the structural, physical, chemical and sensory properties of foods (Kuntz & Kauzmann, 1974). In fact, as reported

Table 2
Microstructural and textural parameters of dough and bread samples.

Dough tension test					Bread compression test
Samples	F_{max} (N)	$Strain_{max}$ (%)	F_{break} (N)	$Strain_{break}$ (%)	$F_{50\%}$ (N)
CTRL S	$0.339^a \pm 0.04$	$32.98^a \pm 4.4$	$0.166^a \pm 0.02$	$45.26^a \pm 5.1$	$6.17^a \pm 1.39$
CTRL P	$0.487^b \pm 0.03$	$13.92^b \pm 1.2$	$0.243^b \pm 0.01$	$18.73^b \pm 1.6$	$9.45^b \pm 0.79$
P-0.4	$0.264^c \pm 0.02$	$18.08^b \pm 1.8$	$0.133^c \pm 0.01$	$24.75^c \pm 1.7$	$4.80^a \pm 0.87$
P-0.7	$0.146^d \pm 0.01$	$22.5^c \pm 1.6$	$0.073^d \pm 0.01$	$32.37^d \pm 2.9$	$4.60^a \pm 0.58$
P-1.0	$0.12^d \pm 0.01$	$16.29^b \pm 1.8$	$0.064^d \pm 0.01$	$23.51^{b,c} \pm 2.2$	$6.60^a \pm 1.30$
Samples	POV (%)	OSVR (1/μm)	FI (1/μm)	St.Th (μm)	St.Sp (μm)
Geometric parameters of dough samples					
CTRL S	$10.76^a \pm 1.5$	$0.0213^a \pm 0.002$	$0.003^{a,b} \pm 0.005$	$294.17^a \pm 34.18$	$794.73^b \pm 86.79$
CTRL P	$35.07^b \pm 8.9$	$0.0119^b \pm 0.001$	$0.004^{a,b} \pm 0.002$	$588.34^b \pm 78.17$	$517.36^a \pm 72.89$
P-0.4	$19.49^a \pm 2.5$	$0.0136^b \pm 0.002$	$0.006^b \pm 0.0001$	$509.78^b \pm 20.64$	$722.61^b \pm 28.31$
P-0.7	$37.02^b \pm 8.9$	$0.0125^b \pm 0.002$	$-0.001^a \pm 0.002$	$488.30^b \pm 73.74$	$452.81^a \pm 34.78$
P-1.0	$23.08^a \pm 6.8$	$0.015^b \pm 0.003$	$0.004^{a,b} \pm 0.001$	$530.67^b \pm 89.55$	$565.91^a \pm 84.52$
Geometric parameters of bread samples					
CTRL S	$44.85^{a,b} \pm 9.6$	$0.0085^a \pm 0.001$	$0.001^a \pm 0.001$	$801.71^a \pm 133.67$	$433.49^a \pm 68.79$
CTRL P	$33.48^a \pm 8.5$	$0.0122^b \pm 0.001$	$0.003^a \pm 0.003$	$521.96^b \pm 59.16$	$443.36^a \pm 72.38$
P-0.4	$48.66^c \pm 5.7$	$0.0074^a \pm 0.001$	$0.001^a \pm 0.001$	$802.08^a \pm 130.88$	$427.04^a \pm 25.16$
P-0.7	$40.59^{a,b} \pm 3.6$	$0.008^a \pm 0.001$	$0.002^a \pm 0.001$	$760.25^a \pm 75.65$	$490.47^a \pm 35.27$
P-1.0	$41.95^{a,b} \pm 6.0$	$0.008^a \pm 0.001$	$0.002^a \pm 0.001$	$745.43^a \pm 55.67$	$487.97^a \pm 55.07$

$^{a-d}$Mean in the same column followed by different superscript letters differs significantly ($p < 0.05$).
Ten specimens were used for each analysis.

by Skendi, Papageorgiou, and Biliaderis (2010), water plays an important role in determining the viscoelastic properties of dough due to its influence on the development of the gluten protein network.

3.1.2. Crumb texture analysis

Compression tests were performed on the crumb of the investigated bread samples in order to evaluate their firmness. For this purpose, the force required to compress the bread sample to 50% of its initial height was taken as a reference for expressing the bread hardness. Results are illustrated in Table 2. As can be inferred from the above table, the highest value for firmness was recorded for the CTRL P sample if compared to the other samples. Moreover, the firmness of the reference sample CTRL S was not significantly different from that of samples in which the yellow pepper flour and durum wheat semolina were separately hydrated (P-0.4, P-0.7, P-1.0). In particular, the latter samples appeared softer than CTRL P sample as demonstrated by the lower resistance to compression. These results seem to be strictly related with those reported in the previous section for dough samples; in fact, crumb from well hydrated dough will require less force to be deformed and it will also be more soft. This finding differs from what was observed in a previous work (Mastromatteo et al., 2012b) and it is probably attributable to the different drying process used to manufacture the vegetable flour used in this work. As seen previously, the addition of water had a strong influence on dough and bread mechanical properties. In addition, the competition for water between durum wheat and yellow pepper flours was avoided through the use of hydrated vegetable flour. In fact, the proposed method seems to promote the formation of a better dough structure.

3.2. Microstructure analysis

Table 2 shows the average values obtained for the four tomographical parameters, POV, OSVR, FI, St.Th and St.Sp, and the results of the statistical analysis for the dough and bread crumb samples respectively. The percentage object volume, i.e. the geometric parameter POV, was calculated for each image as a representation of the percentage total pore content within the sample. As can be noted for the dough samples, the sample CTRL S has the lowest POV value although it is also statistically equal to the samples containing hydrated yellow pepper flour, P-0.4 and P-1.0. Whereas the samples CTRL P and P-0.7 have the highest POV values that are also statistically equal. With regards to the bread crumb samples, also seen in Table 2, the statistical analysis confirms that the sample CTRL S and all the samples containing hydrated yellow pepper flour have POV values that are statistically equal. On the other hand the sample P-0.4 containing hydrated yellow pepper flour has the highest and most significantly different POV value. The results therefore suggest that the addition hydrated yellow pepper flour has no effect on the porosity of the final bread product in relative to that of the CTRL S sample, whereas the addition of the no-hydrated yellow pepper flour causes a significant decrease in porosity in the bread samples. The parameter OSVR indicates the ratio of the surface of the cell walls to the total volume of the object (i.e. dough or bread crumb) and is inversely proportional to St.Th, the average diameter of the pores present in the sample. With regards to the dough samples, sample CTRL S has the highest OSVR value and the lowest St.Th, therefore indicating that there is a wider distribution of smaller pores present in the dough of the CTRL S sample. Whereas the other dough samples have statistically equal lower OSVR and St.Th values therefore indicating a wider distribution of larger pores. Therefore it can be stated that the addition of no-hydrated or hydrated yellow flour does not affect to a great extent the pore size distribution of the dough samples. On the other hand, with regards to the bread samples it can be noted overall that there is a general decrease of the OSVR values and therefore an increase of the St.Th values. These results indicate a general increase in diameter of the pores after baking and therefore a decrease in the pore surface to volume ratio. Although it can be noted that for dough and bread crumb sample added with dehydrated flour, CTRL P, there are no significant changes in the OSVR and St.Th values, this could be due to the fact that water plays an important role in the leavening process and it was lacking in the CTRL P sample. St.Sp is the average distance between the pores, as it can be noted from the table that there is more or less a general decrease of the St.Sp values before and after the baking process. This indicates that in general as the pore size increases (St.Th), the distance between the pores also decreases. With regards to FI parameter, i.e. the index of connectivity and therefore a measure of relative convexity or concavity of the total pore surface, based on the principle that concavity indicates connectivity, and convexity indicates isolated disconnected structures (Lim & Barigou, 2004). A lower FI signifies pores that are connected to each other and has a negative index, on the other hand a higher FI indicates a more disconnected structure (i.e. pores that are distinctly separated from each other) and has a positive index. As it can be noted for all the samples in both groups of studies, the FI is positive for all samples except for sample P-0.7 of the dough samples. This suggests that in general there is a higher percentage of isolated pores present in all the samples that are also convex in structure.

3.3. Sensory analysis

The organoleptic properties of the five investigated bread samples were evaluated by means of a sensory analysis. Results, listed in Table 3, highlighted that the maximum score of overall quality (up to 7.6) was obtained by the reference sample CTRL S. Moreover, also the P-0.7 sample shown a very positive value of this parameter; in fact, there is no statistically significant difference between the abovementioned samples. In particular, with the exception of the sensory attributes such as color and appearance, in which the presence of vegetable flour seems to

Table 3
Sensory characteristics of the investigated bread samples.

	Color	Taste	Appearance	Crust firmness	Crumb firmness	Large bubbles	Overall quality
CTRL S	$7.30^{a} \pm 0.45$	$7.00^{a} \pm 0.35$	$7.40^{a} \pm 0.22$	$6.90^{a,b} \pm 0.42$	$7.80^{a} \pm 0.27$	$7.80^{a} \pm 0.45$	$7.60^{a} \pm 0.42$
CTRL P	$6.20^{b,c} \pm 0.57$	$3.90^{b} \pm 0.22$	$4.20^{b} \pm 0.27$	$5.90^{c} \pm 0.42$	$3.60^{b} \pm 0.42$	$4.20^{b} \pm 0.57$	$3.90^{b} \pm 0.42$
P-0.4	$6.40^{a,b} \pm 0.42$	$5.00^{c} \pm 0.35$	$4.75^{b} \pm 0.27$	$6.10^{b,c} \pm 0.42$	$4.70^{c} \pm 0.27$	$5.30^{c} \pm 0.45$	$5.40^{c} \pm 0.22$
P-0.7	$6.70^{a,b} \pm 0.57$	$6.30^{a} \pm 0.57$	$7.10^{a} \pm 0.42$	$7.00^{a} \pm 0.50$	$6.50^{d} \pm 0.35$	$7.30^{a} \pm 0.27$	$7.40^{a} \pm 0.22$
P-1.0	$5.40^{c} \pm 0.42$	$4.50^{b,c} \pm 0.35$	$4.50^{b} \pm 0.50$	$5.00^{d} \pm 0.50$	$3.80^{b} \pm 0.22$	$5.90^{c} \pm 0.22$	$4.50^{b} \pm 0.35$

$^{a-e}$Mean in the same column followed by different superscript letters differs significantly ($p < 0.05$).
Ten trained tasters and 9-point scale: 1 = extremely unpleasant, 9 = extremely pleasant, 5 = sensory acceptability threshold.

affect the bread quality, the other considered attributes showed similar values for both samples. Moreover, the taste of roasted pepper made the bread pleasant. However, it is worth noting that the P-0.7 sample showed a soft and porous crumb with the presence of very large bubbles related not only to the method of hydration of the vegetable flour, but also because of the proper amount of water added to the formulation. Samples in which yellow pepper flour had different degree of hydration were much different from each other. In particular, the sample with the lowest hydration level (P-0.4) showed just acceptable characteristics due principally to the compact crumb with a presence of smaller bubbles and to a higher crust firmness, which is most probably related to the lower amount of water added. Furthermore, due to the reduced quantities of water used to hydrate the vegetable flour, in the crumb there were dried grains of flour that were not adequately hydrated. Between P-1.0 sample, with the greatest hydration level, and the reference sample CTRL P, obtained using no-hydrated yellow pepper flour, there was no statistically significant difference in terms of overall quality. CTRL P sample showed high crust firmness and compact crumb, which was characterized by the presence of smaller bubbles. Similarly, inside the P-1.0 sample crumb the presence of few small bubbles was observed. Most probably, unlike CTRL P sample, in the case of P-1.0 sample the excessive amount of water used to hydrate the vegetable flour caused the collapse of the structure on itself. This was the cause of a lower loaf volume and a sticky crumb, which, most likely, caused an unpleasant taste.

It can be concluded that the addition of pre-hydrated yellow pepper flour to bread formulation can improve the mechanical as well as the sensory properties of fortified bread only when the right amount of water is used.

3.4. Determination of carotenoids content

As reported beforehand, in this work yellow pepper flour obtained with a low temperature drying cycle was used for the bread production. In fact, Padalino et al. (2013) in their work on the production of yellow pepper flour enriched pasta found that the use of high temperature drying cycle process caused damages to the finished product. Specifically, analytical investigations carried out by Padalino et al. (2013) highlighted that the concentration of Zeaxantin, Lutein and Beta Carotene into yellow pepper flour produced by using the low temperature drying cycle was higher than that of the same flour manufactured at high temperature. This is reflected on the final product in which the characteristics of the raw material are better safeguard.

To prove the preservation of the antioxidant content in the developed functional bread, the concentrations of above mentioned carotenoids were measured in the different steps of the production process: in the dough at the end of leavening and in the products after cooking.

Table 4 shows the results obtained for carotenoids in each food matrix investigated in this work before and after cooking, along with both reference samples, CTRL S and CTRL P. Considering the carotenoid content values, before and after cooking, it can be stated that there were no significant losses of antioxidant activity due to the cooking process. Also in this case, as already observed by Padalino et al. (2013), it is reasonable to assume that the solvent used for the analytical investigations can affect the extraction of carotenoids from different matrices. Therefore, this may be the cause of the slight differences observed in the amount of antioxidant of the investigated samples.

Table 4
Determination of carotenoids content of the dough and bread samples.

Samples	Zeaxanthin (mg/kg)	Capsanthin (mg/kg)	Lutein (mg/kg)	Beta-carotene (mg/kg)
Dough samples				
CTRL S	0.65 ± 0.01^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
CTRL P	5.56 ± 0.28^{b}	0.96 ± 0.04^{b}	1.96 ± 0.13^{b}	0.56 ± 0.03^{b}
P-0.4	3.70 ± 0.15^{c}	0.53 ± 0.02^{b}	0.55 ± 0.06^{c}	0.54 ± 0.04^{b}
P-0.7	3.03 ± 0.20^{d}	$0.33 \pm 0.06^{a,b}$	0.31 ± 0.03^{d}	0.32 ± 0.02^{c}
P-1.0	4.73 ± 0.21^{e}	0.74 ± 0.44^{b}	1.05 ± 0.12^{e}	0.68 ± 0.01^{d}
Bread samples				
CTRL S	0.75 ± 0.00^{a}	0.00 ± 0.00^{a}	0.03 ± 0.01^{a}	0.00 ± 0.00^{a}
CTRL P	6.08 ± 0.18^{b}	1.11 ± 0.08^{b}	2.07 ± 0.08^{b}	0.91 ± 0.07^{b}
P-0.4	3.60 ± 0.39^{c}	0.59 ± 0.10^{c}	0.74 ± 0.07^{c}	0.75 ± 0.05^{c}
P-0.7	4.49 ± 0.21^{d}	0.87 ± 0.07^{d}	0.73 ± 0.08^{c}	0.76 ± 0.05^{c}
P-1.0	4.57 ± 0.19^{d}	0.67 ± 0.03^{c}	1.12 ± 0.08^{d}	1.05 ± 0.04^{d}

a–e Mean in the same column followed by different superscript letters differs significantly ($p < 0.05$).
Analyses carried out in triplicate.

3.5. In vitro digestion

Table 5 shows the effect of reducing sugars during *in vitro* digestion of bread enriched with either pre-hydrated or no-hydrated yellow pepper flour in the ileal phase. All samples analyzed showed rapid starch digestion in presence of pancreatin during the first 20 min, after which the rate of digestion decreased. Moreover, no significant difference in glucose release rates was observed at 60 and 120 min of digestion. Afterward, at the end of the digestion (180 min) only the CTRL S sample recorded a highest release of glucose. In fact, the lowest values of glucose release were observed in all bread samples added with yellow pepper flour, with or without hydration. No significant statistical difference was observed among the above-mentioned samples. This finding suggests that the reducing glucose release is due to the presence of the yellow pepper flour added to the bread and not to its hydration level.

4. Conclusions

In this study, yellow pepper flour was blended to durum wheat semolina to produce fortified bread. The manufactured bread was analyzed to evaluate its physical characteristics, sensorial properties and antioxidant activities as affected by the vegetable flour hydration level. Results showed that the middle hydration level (0.7 L) could be used in the bread formulation without any significant interference with the sensory quality of the bread. Furthermore, the incorporation of yellow pepper flour markedly increased the carotenoids content and therefore the antioxidant activity of the bread. Additionally, the lowest values of glucose release were observed in all bread samples added with yellow pepper flour, with or without hydration, compared to the CTRL S sample. It can be

Table 5
Determination of the *in vitro* glycemic response of the bread samples.

Digestion time (min)	Glucose release (mg/g)				
	CTRL S	CTRL P	P-0.4	P-0.7	P-1.0
0	$95^{a} \pm 8$	$107^{a,b} \pm 3$	$101^{a} \pm 8$	$117^{b,c} \pm 6$	$123^{c} \pm 12$
20	$234^{a,b} \pm 11$	$249^{b} \pm 19$	$185^{c} \pm 8$	$230^{a,b} \pm 12$	$222^{a} \pm 9$
60	$299^{a} \pm 36$	$315^{a} \pm 7$	$279^{a} \pm 13$	$293^{a} \pm 12$	$277^{a} \pm 28$
120	$366^{a} \pm 26$	$343^{a} \pm 28$	$336^{a} \pm 28$	$342^{a} \pm 7$	$323^{a} \pm 11$
180	$394^{a} \pm 21$	$353^{b} \pm 11$	$352^{b} \pm 35$	$349^{b} \pm 13$	$335^{b} \pm 13$

a–c Mean in the same row followed by different superscript letters differs significantly ($p < 0.05$).
Analyses carried out in triplicate.

concluded that hydrated yellow pepper flour at a concentration of 25% can be effectively incorporated in bread to enhance the concentration of functional components with antioxidant properties.

References

Chillo, S., Ranawana, D. V., & Henry, C. J. K. (2011). Effect of two barley β-glucan concentrates on in vitro glycaemic impact and cooking quality of spaghetti. *LWT – Food Science and Technology, 44*, 940–948.

Deshpande, S. S., Rangnekor, P. D., Sathe, S. K., & Salunkhe, D. K. (1983). Functional properties of wheat-bean composite flours. *Journal of Food Science, 48*, 1659–1663.

El-Soukkary, F. A. H. (2001). Evaluation of pumpkin seed products for bread fortification. *Plant Foods for Human Nutrition, 56*, 365–384.

Fan, L., Zhang, S., Yu, L., & Ma, L. (2006). Evaluation of antioxidant property and quality of breads containing *Auricularia auricula* polysaccharide flour. *Food Chemistry, 101*, 1158–1163.

FAO/WHO. (1998). *Report of a joint FAO/WHO expert consultation Rome, 14–18 April 1997*.

Giami, S. Y., Mepba, H. D., Kiin-Kabari, D. B., & Achinewhu, S. C. (2003). Evaluation of the nutritional quality of breads prepared from wheat-fluted pumpkin (*Telfairia occidentalis* Hook) seed flour blends. *Plant Foods for Human Nutrition, 58*, 1–8.

Hathorn, C. S., Biswas, M. A., Gichuhi, P. N., & Bovell-Benjamin, A. C. (2008). Comparison of chemical, physical, micro-structural, and microbial properties of breads supplemented with sweetpotato flour and high-gluten dough enhancers. *LWT – Food Science and Technology, 41*, 803–815.

Hidalgo, A., Brandolini, A., & Pompei, C. (2010). Carotenoids evolution during pasta, bread and water biscuit preparation from wheat flours. *Food Chemistry, 121*, 746–751.

Holmes, G. J., & Kemble, J. M. (2009). *Vegetable crop handbook*. Southeastern US.

Kuntz, I. D., & Kauzmann, W., Jr. (1974). Hydration of proteins and polypeptides. In C. B. Anfinsen (Ed.), *Advances in protein chemistry* (pp. 239–345). New York: Academic Press.

Laverse, J., Mastromatteo, M., Frisullo, P., & Del Nobile, M. A. (2011). X-ray microtomography to study the microstructure of cream cheese-type products. *Journal of Dairy Science, 94*, 43–50.

Lim, K. S., & Barigou, M. (2004). X-ray micro-tomography of cellular food products. *Food Research International, 37*, 1001–1012.

Mansour, E. H., Dworschak, E., Pollhamer, Z. S., Gergely, A., & Hovari, J. (1999). Pumpkin and canola seed proteins and bread quality. *Acta Alimentaria, 28*, 59–70.

Mastromatteo, M., Danza, A., Guida, M., & Del Nobile, M. A. (2012a). Formulation optimization of vegetable flour-loaded functional bread. Part I: Screening of vegetable flours and structuring agents. *International Journal of Food Science and Technology, 47*, 1313–1320.

Mastromatteo, M., Danza, A., Guida, M., & Del Nobile, M. A. (2012b). Formulation optimization of vegetable flour loaded functional bread. Part II: Effect of the flour hydration on the bread quality. *International Journal of Food Science and Technology, 47*, 2109–2116.

Mateos, R. M., LeÛn, A. M., Sandalio, L. M., GÛmez, M., del RÌo, L. A., & Palma, J. M. (2003). Peroxisomes from pepper fruits (*Capsicum annum* L.): purification, characterization and antioxidant activity. *Journal of Plant Physiology, 160*, 1507–1516.

Padalino, L., Mastromatteo, M., Lecce, L., Cozzolino, F., & Del Nobile, M. A. (2013). Manufacture and characterization of gluten-free spaghetti enriched with vegetable flour. *Journal of Cereal Science, 57*, 333–342.

Petitot, M., Boyerb, L., Minierb, C., & Valérie Micard, V. (2009). Fortification of pasta with split pea and faba bean flours: pasta processing and quality evaluation. *Food Research International, 43*, 634–641.

Ptitchkina, N. M., Novokreschonovaa, L. V., Piskunova, G. V., & Morris, E. R. (1998). Large enhancements in loaf volume and organoleptic acceptability of wheat bread by small additions of pumpkin powder: possible role of acetylated pectin in stabilising gas-cell structure. *Food Hydrocolloids, 12*, 333–337.

Ranga Rao, G. C. P., Haridas, R. A. O., Kumar, G. V., & Shurpalekar, S. R. (1980). Utilization of wheat germ in the perpetration of bread and biscuits. *Journal of Food Technology, 17*, 171–175.

Skendi, A., Papageorgiou, M., & Biliaderis, C. G. (2010). Influence of water and barley [beta]-glucan addition on wheat dough viscoelasticity. *Food Research Infernational, 43*, 57–65.

Spies, R. (1997). *Dough rheology and baked product texture*. Huston, USA: CBS Press.

Sun, T., Xu, Z., Wu, C. T., Janes, M., Prinyawiwatkul, W., & No, H. K. (2007). Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum* L.). *Journal of Food Science, 72*, 98–102.